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(54) Title: THE USE OF HEPARIN OR HEPARAN SULPHATE IN COMBINATION WITH CHITOSAN FOR THE PREVENTION OR TREATMENT OF INFECTIONS CAUSED BY HERPES VIRUS

(57) Abstract

The use of heparin or heparan sulphate in combination with chitosan for the manufacture of a medicament for the prevention or treatment of infections caused by herpes virus in mammal including man; and a process for the treatment of infectious diseases caused by herpes viruses.

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THE USE OF HEPARIN OR HEPARAN SULPHATE IN COMBINATION WITH CHITOSAN FOR THE PREVENTION OR TREATMENT OF INFECTIONS CAUSED BY HERPES VIRUS

TECHNICAL AREA

The present invention refers to the use of certain sulphated polyanionic glucoseaminoglycans for the manufacture of medicaments that can be used for preventing or treating infections caused by herpes virus.

BACKGROUND OF THE INVENTION

Herpes is the common name for a family of viruses which generate infectious disease in mammals including man. Contrary to the majority of infections herpes viruses usually generate what is called latent infections, thus residing dormant in nerve roots of the central nervous system and subsequently causing active disease when activated by some unknown factor. There is today no method available for the treatment of such infections. Factors activating the disease are mostly unknown but it is believed that stress, fever, menstruation, sunlight etc. can be the cause of activation of the virus. From a practical viewpoint the herpes viruses typically cause a skin rash as a major manifestation.

Two main types of herpes simplex viruses are known, HSV-1 causing oral herpes and HSV-2 causing genital herpes. The viruses cause reoccurring, painful skin and mucosa lesions in about 10% of the population. The pain and cosmetic effects of the disease are of primary concern for most patients and pain relief and more rapid healing would represent a major advance in the art. It is essential to note that healing occurs spontaneously in most patients and one major object of the effort should therefore be directed to hasten the healing process and to provide analgesic effect.

SUMMARY OF THE INVENTION

The present invention has for its main object to provide for new techniques for the prevention or treatment of infection disease caused by herpes virus in mam-

mals including man.

Another object of the invention is to provide for a new combination of active ingredients to be used for the manufacture of a medicament for such prevention or treatment.

Yet another object of the invention is to provide a process for the treatment of infections in mammals including man caused by herpes virus.

For these and other objects which will be clear from the following disclosure the invention provides for new techniques residing in the use of heparin or heparan sulphate in combination with chitosan for the manufacture of a medicament for the prevention or treatment of infectious diseases caused by herpes virus in mammals including man.

In such use it is preferred that the manufacture of said medicament is based on a combination of heparin and chitosan.

Both heparin and heparan sulphate are commercially available on the market from several manufacturers. Also partially hydrolyzed forms of these ingredients can be used provided that their biological activity remains substantially unaltered.

The medicament to be used to prevent or combat infectious disease caused by herpes viruses can be presented in different physical forms, for example as powders, ointments, pastes, gels, suspensions or solutions. The form to be used is, of course, adapted to the nature of the disorder to be treated.

One of the main components in the present medicament is the polysaccharide chitosan which is a linear 1,4-bound polysaccharide built up from β-D-glucoseamine entities. The chitosan is manufactured by N-deacetylation of chitin, a polymer which forms the shell of inter alia insects and crayfish. Commercial chitin is recovered from crab and shrimp shells which constitute waste products from the fishing industry. By regulating the alkali

treatment of chitins it is possible to manufacture chitosans of varying degrees of N-acetylation. When treating chitin with alkali, such as sodium hydroxide, N-deacetylation takes place wherein acetamido groups are converted to amino groups thus forming chitosan.

The physical properties of chitosan affecting its utility depend on the degree of N-acetylation, molecular weight and homogeneity. Chitosan is biodegradable, both by chitinas from the digestive system and by lysozyme in body fluids.

It is preferred in connection with the use of the present invention that the chitosan has a degree of N-acetylation of at most about 90% and preferably at most about 50%. It is particularly preferred that the degree of N-acetylation is less than about 25%.

The two main components of the medicament involved in the present invention, heparin or heparan sulphate and chitosan, are suitably used in combination with conventional carriers or excipients of a medicinally acceptable character. Quite generally it is preferred that the matrix is an aqueous matrix, and the carrier or the excipient may contain a viscosity-increasing polysaccharide, which can be selected from hemicelluloses, for example arabino xylanes and glucomannanes, plant gums, for example guar gum, locust bean gum, celluloses and derivatives thereof, for example methylcellulose, ethylcellulose, hydroxiethylcellulose, carboximethylcellulose, starch and starch derivatives, for example hydroxiethylstarch or crosslinked starch, microbial polysaccharides, for example xanthan gum, curdlan, pullulan, dextran. Also algi polysaccharides, for example agar, carrageenans, alginic acid, can be used as a constituent in the carrier or excipient.

A preferred polysaccharide for use in the carrier or excipient is a cellulose derivative, for example methylcellulose.

In the subject medicament it is preferred that the

combined amount of viscosity-increasing polysaccharide and said chitosan is less than about 10% by weight based on the total weight of the medicament.

A preferred physical form of the medicament is the gel form, and in such gel-formed medicament said combined amount of polysaccharide and chitosan is preferably within the range about 0,5 to about 5% by weight based on the medicament as a whole.

Said heparin or heparan sulphate is preferably present in the medicament in an amount of from about 0,1 to about 2% by weight.

The present invention also provides for a process for the prevention or treatment of infectious diseases in mammals including man caused by herpes viruses. This process comprises the step of applying onto a site in need of treatment an active amount of a medicament containing in combination heparin or heparan sulphate and chitosan.

EXAMPLES OF EMBODIMENTS

The present invention will in the following disclosure be illustrated in connection with non-limiting examples. In said examples parts and percentages refer to weight if not otherwise stated. This illustration is made in association with the appended drawing, wherein:

The effect of HSV-1 plaques has been plotted against serial sample dilution for 5 different compositions illustrating complete medicament and compositions excluding one or more constituents.

EXAMPLE 1 - Preparation of complete medicament

The hydrochloride salt of chitosan having an N-deacetylation degree of 16% (Pronova Biopolymers, Drammen, Norway) is dissolved in sterile filtered distilled water to form a 3% solution of the chitosan.

A 0,6% solution of native heparin containing methylcellulose in a concentration of 4 g/L is prepared.

Both solutions are autoclaved and admixed under ste-

rile conditions in equal parts. Sorbic acid is added as a preservative in an anti-fungal amount.

EXAMPLE 2

The composition of Example 1 is prepared in a similar manner but excluding the sorbic acid.

EXAMPLE 3

The composition of Example 1 is prepared in a similar manner but excluding sorbic acid and heparin.

EXAMPLE 4

The composition of Example 1 is prepared in a similar manner but this time excluding sorbic acid and chitosan.

EXAMPLE 5

The composition of Example 1 is prepared in a similar manner but this time excluding, in addition to sorbic acid and heparin, also chitosan.

EXAMPLE 6 - Experimental procedure

The diluent used in the experiments is isotonic NaCl (0.136 M) and Eagle Minimum Essential Medium (EMEM). The virus used is HSV-1, strain KOS321.

The samples are diluted in 24 well plates. 1 mL of sample is mixed with 1 mL of isotonic NaCl and then serial 2-fold dilutions in isotonic NaCl up to a dilution of 1:32 are performed. The last well in each row contains only isotonic NaCl. The same solutions are also made in EMEM instead of isotonic NaCl.

Detailed procedure:

1. Add 100 PFU of HSV-1 in 100 µL of isotonic NaCl to serial dilutions of sample.
2. Incubate for 10 min at RT.
3. Wash the cells (Green monkey kidney cells (GMK AH1), confluent monolayers, 3 days old, 6 well plates) 1 x with

2 mL of either isotonic NaCl or EMEM.

4. Add 1 mL of virus-sample mixture to cells.

5. Incubate for 1h at RT.

6. Wash the cells 2 x with 2 mL of EMEM.

7. Add 3 mL of standard methylcellulose solution.

8. Incubate for 3 days in Co_2 incubator at 37°C.

9. Stain the cells with crystal violet solution and count the viral plaques.

RESULTS

The appended drawing shows by way of diagram the results as HSV-1 plaques plotted against the sample dilution (neg. log2). It can be seen from the results illustrated in the drawing that a dilution of 1:2 and 1:4 resulted in inhibition by all preparations probably due to the presence of methylcellulose. In dilutions 1:16 and 1:32 only the preparations containing heparin are active. Up to a dilution of 1:32 the inhibitory effect of the preparations containing both heparin and chitosan was maintained.

Test for toxicity have shown that all compositions were non-toxic vis-à-vis GMK cells.

EXAMPLE 7 - Clinical tests

The following clinical testing was made using a composition in accordance with Example 1 above in the form of a gel. All tests were directed to the prevention or treatment of orolabial infection caused by HSV-1 virus.

Case 1

A 15 year old boy was repeatedly treated, both by prophylactic treatment of early symptoms and treatment of established infection. Local application of the composition of Example 1 eliminated early symptoms within 24 hours and provided healing in 72 hours, respectively.

Case 2

A 48 year old male was treated prophylactically for early

symptoms, the result being the same as in Case 1 above.

Case 3

A 9 year old female has successfully used the medicament of Example 1, both against established disease and in prophylactic treatment.

Case 4

A 3 year old boy having fully developed lip infection was topically treated with the composition of Example 1 above. This resulted in arrested infection in 2 days and healing in 4 days.

Case 5

A 52 year old male has successfully used the gel of Example 1 both for the treatment of established disease and for prophylactic treatment of early symptoms.

The results presented above relating both to inhibition of virus cell growth and clinical testing all verify the astonishing effect the present compositions have on the infectious disease caused by herpes viruses. The fact that such infections could be successfully treated using a combination of heparin and chitosan was in fact unexpected and surprising. Although the invention is not restricted to any particular theory or mechanism of action it may be that the matrix used in the composition provides for slow release of the heparin in an active form. The matrix including chitosan can be considered to bind the heparin by ionic bonds so that it will not be inactivated by enzymes present in the environment of treatment, such as the enzyme heparinas. This was clearly unexpected in view of the sensitivity of heparin to enzymatic degradation and inactivation.

It is to be noted that the invention as exemplified above is not restricted to the presented embodiments since modifications are obvious to the skilled artisan. Therefore, the scope of the invention is only restricted by the appended claims.

CLAIMS

1. The use of heparin or heparan sulphate in combination with chitosan for the manufacture of a medicament for the prevention or treatment of infections caused by herpes virus in mammal including man.

2. The use according to claim 1, wherein the manufacture is based on the combination of heparin and chitosan.

3. The use according to claim 1 or 2, wherein the chitosan has a degree of N-acetylation of at most about 90% and preferably at most about 50%.

4. The use according to claim 3, wherein the chitosan has a degree of N-acetylation of less than about 25%.

5. The use according to any one of the preceding claims, wherein said medicament further contains a pharmaceutically acceptable carrier or excipient.

6. The use according to any one of the preceding claims, wherein the medicament is in the form of a powder, an ointment, a paste, a gel, a suspension, a solution or a film.

7. The use according to claim 5 or 6, wherein said carrier or excipient comprises a viscosity-increasing polysaccharide.

8. The use according to claim 7, wherein said viscosity-increasing polysaccharide is constituted by a cellulose derivative.

9. The use according to any one of the preceding claims, wherein said medicament comprises an aqueous matrix.

10. The use according to any one of claims 6 to 8, wherein said viscosity-increasing polysaccharide together with said chitosan is present in said medicament in an amount of less than about 10% by weight.

11. The use according to claim 10, wherein said medicament is in the form of a gel.

12. The use according to claim 11, wherein said

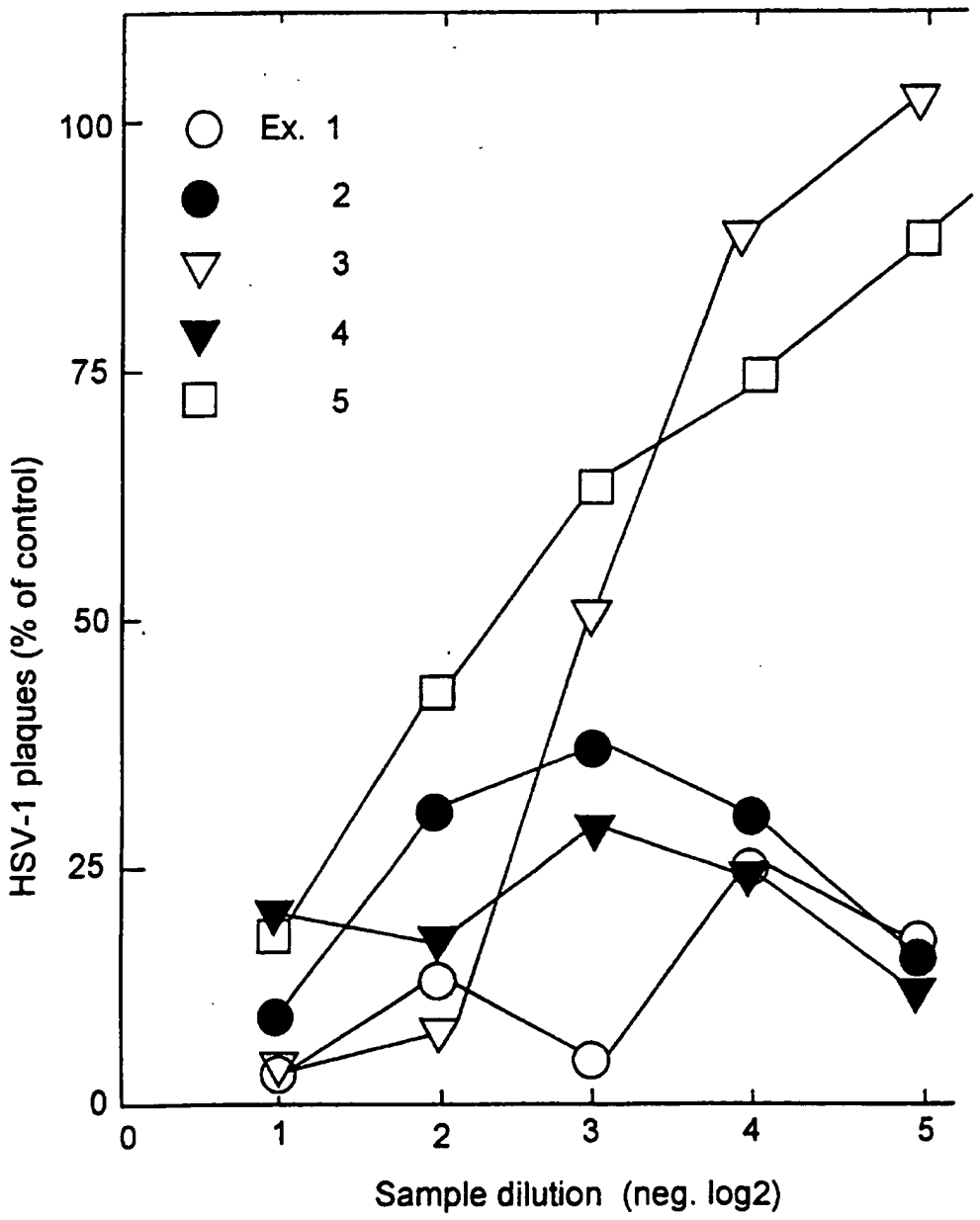
viscosity-increasing polysaccharide together with said chitosan is present in said medicament in an amount of from about 0.5 to about 5% by weight.

13. The use according to any one of the preceding claims, wherein said heparin or heparan sulphate is present in the medicament in an amount of from about 0.1 to about 2% by weight.

14. A process for the prevention or treatment of infections in mammals including man caused by herpes virus comprising the step of applying onto a site in need of treatment an active amount of a medicament containing in combination heparin or heparan sulphate and chitosan.

15. A process according to claim 14, wherein said medicament contains heparin and chitosan.

16. A process according to claim 14 or 15, wherein the medicament is in the form of a powder, an ointment, a paste, a gel, a suspension, a solution or a film.


Ex. 1
2
3
4
5
100
75
50
25
0
0
1
2
3
4
5
HSV-1 plaques (% of control)
Sample dilution (neg. log2)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 97/01320

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 31/725, A61K 31/73
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0497341 A2 (FARMITALIA CARLO ERBA S.R.L.), 5 August 1992 (05.08.92) --	1-13
A	EP 0240098 A2 (KABUSHIKI KAISHA UENO SEIYAKU), 7 October 1987 (07.10.87) --	1-13
A	EP 0000133 A1 (CIBA-GEIGY AG), 10 January 1979 (10.01.79) --	1-13

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 November 1997	18 -11- 1997
Name and mailing address of the ISA/ Swedish Patent Offic Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Eva Johansson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 97/01320

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 14-16
because they relate to subject matter not required to be searched by this Authority, namely:
See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/10/97

International application No.
PCT/SE 97/01320

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0497341 A2	05/08/92	AU 660002 B	08/06/95
		AU 1052492 A	06/08/92
		CA 2060277 A	01/08/92
		DE 69115717 D,T	02/05/96
		EP 0560835 A,B	22/09/93
		SE 0560835 T3	
		JP 6080583 A	22/03/94
		JP 6504942 T	09/06/94
		PL 168480 B	29/02/96
		US 5288704 A	22/02/94
		US 5402943 A	04/04/95
		DE 69200538 D,T	02/03/95
		EP 0528006 A,B	24/02/93
		SE 0528006 T3	
		GB 2259604 A,B	17/03/93
		US 5379147 A	03/01/95
EP 0240098 A2	07/10/87	AU 597635 B	07/06/90
		AU 7107487 A	08/10/87
		CA 1277239 A	04/12/90
		JP 1151521 A	14/06/89
		JP 1648661 C	13/03/92
		JP 2007577 B	19/02/90
		JP 63045223 A	26/02/88
		OA 8581 A	30/09/88
		PH 25964 A	13/01/92
		US 4840941 A	20/06/89
EP 0000133 A1	10/01/79	AU 522600 B	17/06/82
		AU 3715378 A	20/12/79
		CA 1116083 A	12/01/82
		JP 1419447 C	14/01/88
		JP 54008727 A	23/01/79
		JP 62025126 B	01/06/87
		LU 77562 A	26/03/79
		US 4465666 A	14/08/84
		ZA 7803480 A	25/07/79